Remarks/Arguments

The specification has been amended to delete SEQ ID NOs: 4 and 5. Claims 1, 4-8, 10-11, 13-15, 32 and 35-40 have been rejected. Claims 1, 32, 35, 39 and 40 have been canceled. Claims 4 – 8, 10 – 11, 13 – 15 and 36 – 38 remain pending. Claims 4 – 8 and 10 have been revised to depend from claim 36. Claims 11 and 37 have been amended at the Examiners suggestion to improve claim form. Claim 38 has been amended to more clearly define the invention. No new matter has been added by way of the claim amendments. Reconsideration and withdrawal of the rejections are respectfully requested in light of the following remarks.

Objections and rejections that no longer apply to the specification and claims as amended herein

The following objections and rejections have been rendered moot by amendment:

The Examiner maintained the objection to the amendments that incorporate SEQ ID Nos: 4 and 5 into the specification under 35 U.S.C. 132(a) for introducing new matter into the disclosure and the rejection of claims 1, 4-8, 10-11, 13-15, 32 and 35 under U.S.C. 112, first paragraph as failing to comply with the written description requirement for introducing new matter.

Applicants have deleted SEQ ID NOs: 4 and 5 from the specification and canceled claims 1, 32 and 35.

The Examiner rejected claim 39 under 35 U.S.C. 112, second paragraph, as being indefinite by reference to "GenBank accession number 1771281".

Applicants have canceled claim 39.

The Examiner rejected claim 40 that incorporates SEQ ID NO:3 as a structural limitation for Dab1 proteins under 35 U.S.C. 112, first paragraph for inserting new matter.

Applicants have canceled claim 40.

Applicants maintain that these objections and rejections are improper for reasons set forth in prior responses, but have decided to render them moot by amendment in the interest of furthering prosecution.

Objection to Claims 11 and 37

Claim 11 is objected to for the recitation of "the polypeptide fragment TPAPRQSS(PO4)PSKSSA (SEQ ID NO:3) which contains a phosphate group on the 8th amino acid)". Claim 37 is objected to for the recitation of "QSSPSK (SEQ ID NO:1)" and "SSASHVSDPRADDIFEEGFESPSK (SEQ ID NO:2)" The Examiner suggests replacement of these phrases with the appropriate SEQ ID NO.

Applications have revised claims 11 and 37 to delete the amino acid sequence and recite only the SEQ ID NOs.

Claims 36, 38 and 40 are enabled

The Examiner rejected claims 36, 38 and 40 under 35 U.S.C. 112, first paragraph for lack of enablement. The Examiner maintains that the specification, while being enabling for a method for detecting Cdk5 serine kinase activity by determining whether serine at position 491 or 515 of Dab1 is or is not phosphorylated, wherein Dab1 phosphorylation at position 491 and/or 515 indicates Cdk5 serine kinase activity, does not reasonably provide enablement for the broad scope of claimed methods.

Applicants have canceled claim 40, rendering the rejection of this claim moot.

The Examiner rejected claim 36 as being overly broad. The Examiner asserts Applicants are claiming a method for detecting Cdk5 activity by determining whether any serine of the carboxy terminal domain of Dab1 is phosphorylated. The Examiner rejected claim 38 as being overly broad as a method for detecting Cdk5 activity by detecting binding of a phosphoantibody generated against SEQ ID NO:3 to any serine of the carboxy terminal domain of Dab1. The Examiner states that the methods encompass detection of Dab1 phosphorylation in both an unpurified biological sample, e.g., brain and blood, and biological samples that have been clarified and purified.

Applicants respectfully disagree. Claims 36 and 38 are directed to a method for determining whether the carboxy terminal domain of Dab1 in a biological sample is phosphorylated on a serine within a candidate sequence. The specification clearly defines a candidate sequence as a sequence of amino acids which contains a serine followed by a proline in +1 position and a lysine in +3 position, the serine being a preferred site for Cdk5 activity. Applicants assert the claims only encompass serines in the carboxy region that fall within the definition of a candidate sequence. In the present invention, only

serines 491 and 515 in the carboxy region of murine and human Dab1 fall within the definition of a serine within a candidate sequence (a serine followed by a proline in +1 position and a lysine in +3 position) as claimed and described in the specification.

The Examiner asserts on page 19, 1st paragraph of the Office Action, there are 5 potential phosphorylation sites in Dab1, as described in Niethammer et al., i.e. serines 260, 400, 481, 491 and 515, which are followed by a proline. These are described in Niethammer as consensus phosphorylation sites for cyclin-dependent kinases (cdks), not cdk5 phosphorylation sites. Serines 260 and 481 have a threonine at the +3 position and serine 400 has a serine at the +3 position. Thus, none of these serines, nor any other serine, other than 491 and 515, in the carboxy terminal region of Dab1 falls within the scope of the claims.

The Examiner acknowledges on page 20 of the Office Action that the specification discloses two serine residues at positions 491 and 515 of mouse and human Dab1 that are phosphorylated specifically by Cdk5 and thus indicative of Cdk5 activity. The Examiner acknowledges that the specification is enabling for these two serines. Since these are the only serines in the Dab1 carboxy region that fall within the definition of a candidate sequence, claims 36 and 38 are enabled. For the reasons set forth above, reconsideration and withdrawal of this rejection are respectfully requested.

Claims 6-8 and 36-37 are not obvious

The Examiner asserts that claims 1, 6-8, 36-37 and 39-40 are rejected under 35 U.S.C. 103(a) as being unpatentable over Curran et al. (US Patent 6,323,177;"Curran") in view of Keshvara et al (J.Biol. Chem. 376:16008 – 16014, 2001; "Keshvara"), Niethammer et al. (Neuron 28:697 – 711, 2000; "Niethammer"), and GenBank Accession Numbers 1771281 and 3288851. The Examiner asserts Curran teaches *in vitro* phosphorylation of Dab1 on serine residues, but acknowledges that Curran does not teach that Dab1 is phosphorylated in a biological sample, e.g., brain and blood. The Examiner asserts Niethammer and Keshvara show various methods for analysis of a phosphoprotein. Niethammer teaches a method for determining the sites of phosphorylation of the Cdk5 substrate polypeptide NUDEL. Keshvara teaches a method of identifying sites of tyrosine phosphorylation of Dab1 by Src. GenBank Accession Numbers 1771281 and 3288851

disclose the amino acid sequences of murine and human Dab1, respectively. Given the prior art, the Examiner asserts a skilled artisan would have recognized by visually inspecting the amino acid sequences, five potential Cdk5 serine-proline phosphorylation sites (as noted by Niethammer) are present at positions 260, 400, 481, 491 and 515.

Applicants respectfully disagree. The present invention is based on the discovery that Dab1 is specifically phosphorylated by Cdk5. Before the present invention, a substrate that is selectively phosphorylated by Cdk5 had not been identified. As discussed in more detail above, Niethammer suggests serines 491 and 515 as potential sites for cdk (not just Cdk5) activity. Until Applicants showed in the present invention that Dab1 is an *in vivo* target of Cdk5, one could not have known or predicted that certain sites in Dab1 would only be phosphorylated by Cdk5. Nor does the prior art suggest which serines of Dab1, serines within a candidate sequence (serines 491 and 515), would be a specific target of Cdk5 activity.

Although Curran teaches that Dab1 is phosphorylated by Cdk5 activity in vitro, there is no suggestion in this reference or other prior art references that Cdk5 phosphorylates Dab1 in a biological sample. It is well known in the art that many proteins act as substrates in *in vitro* phosphorylation assays which are not *in vivo* targets for the kinase used in the assay and are not biological targets. Curran only suggests screening for inhibitors and agonists of *in vitro* phosphorylation of Dab1 in connection with reelin binding to VLDLR. Curran does not suggest screening for inhibitors and agonists of *in vivo* Dab1 phosphorylation by Cdk5.

Ohshima et. al. (PNAS Vol. 98, No. 5, 2764-2769, 2001, reference AK2 in the information disclosure) teaches on page 2768, second column: "Although *in vitro* studies indicate that serine/threonine residues(s) of Dab1 can be phosphorylated by Cdk5/p35 kinase, there is no evidence that Cdk5/p35 phosphorylates Dab1 *in vivo*. It has been shown that the Reelin-induced phosphorylation of tyrosine residue(s) of Dab1 is essential for its function. However, the significance of the phosphorylation of serine/threonine residue(s) remains to be investigated. Our present studies indicate the possibility of interactions of Cdk5/p35 kinase and Reelin/Dab1 either in signaling pathways or in indirect regulation of common targets." Thus, the prior art teaches away from direct

phosphorylation of Dab1 by Cdk5 in a biological sample by suggesting the interaction between Cdk5 and Dab1 is via a signaling pathway or indirect regulation.

Niethammer makes no suggestion of Cdk5 phosphorylation of Dab1 in a biological sample when on page 705, second column, in the paragraph headed "Discussion", it is stated there are three pathways that control neuronal migration. One through reelin by phosphorylation of Dab1, another through Cdk5 and a third through LIS1 and PAF. Nothing in this reference suggests that Dab1 is phosphorylated by Cdk5 in a biological sample.

Keshvara teaches *in vitro* and *in vivo* methods for identifying Dab1 phosphorylation by Src. However, there is nothing in Keshvara, Curran or Niethammer to suggest Dab1 phosphorylation by Cdk5 *in vivo*. As discussed above, it is well known in the art that many proteins act as substrates in i*n vitro* phosphorylation assays which are not *in vivo* substrates for that kinase.

On page 707 column 2 of Niethammer, under the heading "NUDEL Is a Phosphoprotein", it is stated that NUDEL has five consensus Cdk (Not Cdk5) phosphorylation motifs. This reference continues with "It is phosphorylated by Cdk5 *in vitro* and in cotransfected cells. Mutagenesis studies suggest that among the five Cdk5 sites, the first three are likely to be phosphorylated by Cdk5." Furthermore, as discussed above and acknowledged by the Examiner, Niethammer describes Cdk5 phosphorylation sites as a serine or threonine followed by a proline. It does not suggest the candidate sequence of the present invention, a serine followed by a proline in the +1 position and a lysine in the +3 position. Therefore Niethammer fails to teach the identity of the "candidate sequence" of the present invention . Furthermore, Niethammer leaves open the possibility of phosphorylation of other kinases on its Cdk phosphorylation sites. Niethammer does not provide substrate sites that indicate specific Cdk5 activity.

Since Niethammer does not teach a "candidate sequence" as claimed, one could not identify serines 491 or 515 as serines within a candidate sequence by visual inspection of the amino acid sequences of Gen Bank Accession Number 1771281 and 3288851.

Claims 1, 39 and 40 have been canceled rendering these rejections moot. Given the arguments above, Applicants assert the combination of Curran, Niethammer, Keshvara and GenBank Accession Numbers 177281 and 3288851 do not render the present invention obvious. Until the present invention, it was not obvious that Dab1 is specifically phosphorylated by Cdk5, nor were the specific phosphorylation sites in Dab1 obvious. Applicants respectfully request reconsideration and withdrawal of the rejection under U.S.C. 103(a) against claims 6-8 and 36-37.

Claims 10 – 11, 13 – 14 and 38 are not obvious

The Examiner rejected claims 10-11, 13-14, 32, 35 and 38 under 35 U.S.C. 103(a) as being unpatentable over Curran in view of Keshvara, Niethammer and GenBank Accession Numbers 1771281 and 3288841 as applied to claims 1, 6-8, 36-37 and 39 – 40 above and further in view of Howell et al.(Genes Develop. 13:633 – 648, 1999; cited as reference AY1 in the IDS filed on 3/25/02; "Howell") , Fu et al. (Nature Neurosci. 4:374-381; "Fu"), Michalewski et al. (Analytical Biochem. 276: 254 – 257, 1999; "Michalewski"), and Zhen et al. (J. Neurosci. 21:9160 – 9167, 2001: "Zhen"). These claims limit the claimed methods to detection of Dab1 phosphorylation using an antibody that binds to Dab1 only when it is phosphorylated on serine or to an antibody generated against SEQ ID NO:3.

Howell teaches a method for analyzing *in vivo* and *in vitro* Dab1 tyrosine phosphorylation using an anti-tyrosine antibody. Fu teaches a method for analyzing Cdk5 serine phosphorylation of ErbB3 using an anti-phosphoserine antibody, Michalewski teaches a polyclonal antiphosphoserine antibody and Zhen teaches a monoclonal antiphosphoserine antibody. The Examiner asserts one would have been motivated to combine the prior art references because Curran expressly teaches that Cdk5 phosphorylates serines of Dab1 and the sites of Cdk5 phosphorylation of Dab1 can be identified and exploited to screen for agonists and antagonists and the use of an anti-phosphoserine antibody to detect phosphoserine avoids the use of radioactivity. One would have had a reasonable expectation of success to analyze the phosphorylation of mouse or human Dab1 at positions 260, 400, 481, 491 and 515 using an anti-phosphoserine antibody.

For the reasons stated above, there is nothing in the prior art references of Curran, Keshvara, Niethammer and the recited GenBank Accession Numbers to suggest the inventions in claims 6-8 and 36-37. Thus combining Howell, Fu, Michalewski and Zhen with Curran, Keshvara, Niethammer and the recited GenBank numbers does not render the antibodies of claims 10 - 11, 13 -14 and 38 obvious.

Applicants have canceled claims 32 and 35 rendering the rejection of these claims moot. Applicants respectfully request the reconsideration and withdrawal of the rejection of claims 10 - 11, 13 - 14 and 38 under U.S.C. 103(a).

Conclusion

It is believed that the objection to the specification and rejections of Claims 4-8, 10-11, 13-15 and 36-38 have been overcome and request allowance of all claims.

It is not believed that extensions of time or fees for net addition of claims are required, beyond those that may otherwise be provided for in documents accompanying this paper. However, in the event that additional extensions of time are necessary to allow consideration of this paper, such extensions are hereby petitioned under 37 CFR § 1.136(a), and any fee required therefore (including fees for net addition of claims) is hereby authorized to be charged to Deposit Account No. 501968.

Respectfully submitted,

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